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Final Report

Title: CULTURAL TECHNIQUES FOR INCREASING THE
ROOT-SHOOT SIZE RATIOS OF CONTAINERIZED
WHITE OAK (QUERCUS ALBA) SEEDLINGS

Project Location: The Ohio Agricultural Research and
Development Center, Wooster, Ohio

Project Leader: Dr. Merlyn M. Larson, OARDC

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Abstract

Cultural treatments of IAA and kinetin seed soaks, radicle prunings, and root prunings were tested alone and in combination on White oak (Quercus alba) seedlings grown in two sizes of paper containers to increase root-shoot ratios. Radicle pruning increased number of main roots, larger containers increased root dry weight, but root-shoot ratios were not increased.

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Introduction

Purpose

The purpose of this report is to present information obtained at The OARDC which shows the effects of different combinations of IAA and kinetin plant hormone treatments, root pruning methods, and container sizes on containerized White oak (Quercus alba) seedling growth parameters and resultant root-shoot size ratios.

Background

The current interest in planting high-value taprooted hardwood species on moisture stressed sites such as reclaimed land, south-facing slopes, and areas of high vegetative competition is hampered by the inability of conventional bare-root nursery stock to adapt to these soil moisture conditions. These seedlings develop limited root systems in the nursery, often consisting of a single main root with few lateral roots, which prevent adequate water absorption in low moisture soils and results in poor seedling growth and survival rates on these planting sites. What is needed, therefore, is a method of producing nursery seedlings with more extensive and fibrous root systems so that they can adapt to moisture stressed sites and maintain a high level of physiological vigor necessary for successful seedling establishment.

Several cultural techniques potentially capable of improving the root development of tree seedlings are known. One of these methods is the growth of seedlings in containers. Containerized seedlings enhance root development by maintaining an individualized and continuous soil environment up through outplanting, by aiding in root shaping, and by providing physical protection to the roots during handling. Another method which could improve root development is the application of indoleacetic acid (IAA) and kinetin (K) plant hormones to the ungerminated seeds or growing seedlings. IAA is a stimulant of lateral root formation and kinetin assists this process by stimulating cellular division. A third method of interest for improving root development is the pruning of seedling radicles after germination, and pruning root tips during later growth, which stimulates multiple root regeneration at the cuts. These three cultural methods; IAA and K hormone treatments, growth in containers, and root and radicle pruning methods, deserve to be tested on taprooted hardwood species, alone and in combination, because of the potential they offer in increasing the root development and root-shoot size ratios of these seedlings.

Previous Work

A study involving the containerization of White ash (Fraxinus americana) showed that seedling growth in paper containers was successful, but no quantification of root development or field performance was made.¹

¹Proceedings of the N. American Containerized Forest Tree Seedling Sym., Tinus, Stein, Balmer, Denver, Co., 1974, p.129-30

A study involving the growth of bare-rooted Black walnut (Juglans nigra) seedlings after radicle prunings and root prunings were performed in the nursery bed, showed that root fibrosity greatly increased but that survival and growth rates were insignificantly greater than those of unpruned seedlings. Pruned seedlings did have slightly greater shoot heights and diameters four years later, however.²

Several studies have been conducted concerning the containerization of taprooted Quercus spp. A comparative study of containerized and bare-root stock of Quercus petraea seedlings showed the container stock to have slower growth rates than the bare-root stock.³ Another comparative study of containerized and bare-root stock of Quercus alba showed the two types of stock to be about equal in growth rates.⁴ A third study comparing the field performances of containerized Quercus alba and Quercus rubra suggested that their low growth rates were due to low root-shoot ratios, and that higher ratios were needed for better field performance.

Project Scope

White oak (Quercus alba) was chosen to be tested on in this project because of seed availability and ease of germination.

²Tree Planters' Notes 23(2): 22-25, "Root Fibrosity Proves Insignificant in Survival, Growth of Black Walnut Seedlings", by Williams, Robert D., 1972.

³Ibid. 1: pp. 129-30.

⁴Ibid. 2: pp. 197-199.

The tests consisted of IAA and K seed soaks, growing the seedlings in two sizes of paper containers to see if a larger container promoted better root development, performing radicle prunings on the germinated seeds, and performing root prunings through the container walls, referred to as container prunings.

Seedling samples were harvested at three intervals during the course of growth and measurements were made on root and shoot size parameters. Data collected from these measurements was analyzed using one-way analyses of variance, and Duncan's Multiple Range Tests to see if there were significant differences between treatment effects on growth parameters.

The following report describes the project design, the experimental procedure, the treatment analyses, and presents summarized data and conclusions.

Section I: Project Design

A. Controlled conditions of seedling growth

1. Seed supply

The seeds used in this project were collected at the Mohican Hills Golf Course near Wooster, Ohio, on September 22, and were stored in plastic bags at 3° C. *† — a seedling in a greenhouse.*

2. Soil medium

The soil medium used for seedling cultivation consisted of a 1:1 ratio of peat moss and vermiculite.

3. Water

The seedlings were watered daily by hand.

4. Temperature

Greenhouse temperature was maintained at 65°F.

5. Light

Artificial light was provided at a daily duration of 16 hours, and at an intensity of 2000±200 foot-candles.

B. Varied treatments

To determine the effects of the different cultural techniques tested, the treatments were apportioned to sets of seedlings as follows.

1. Hormone treatments

One-half of the ungerminated seeds of each species were given the IAA and kinetin seed soak treatment while the other half were given a control soak in water.

2. Radicle prunings

Radicle prunings were conducted on one-half of the germinated seed before planting, while the other half were planted unpruned.

3. Container sizes

One-half of the germinated seeds were planted in paper containers measuring 27cm. x (~~5.0~~^{3.5}cm.)², while the other half were planted in containers measuring 27cm. x (5.0cm.)².

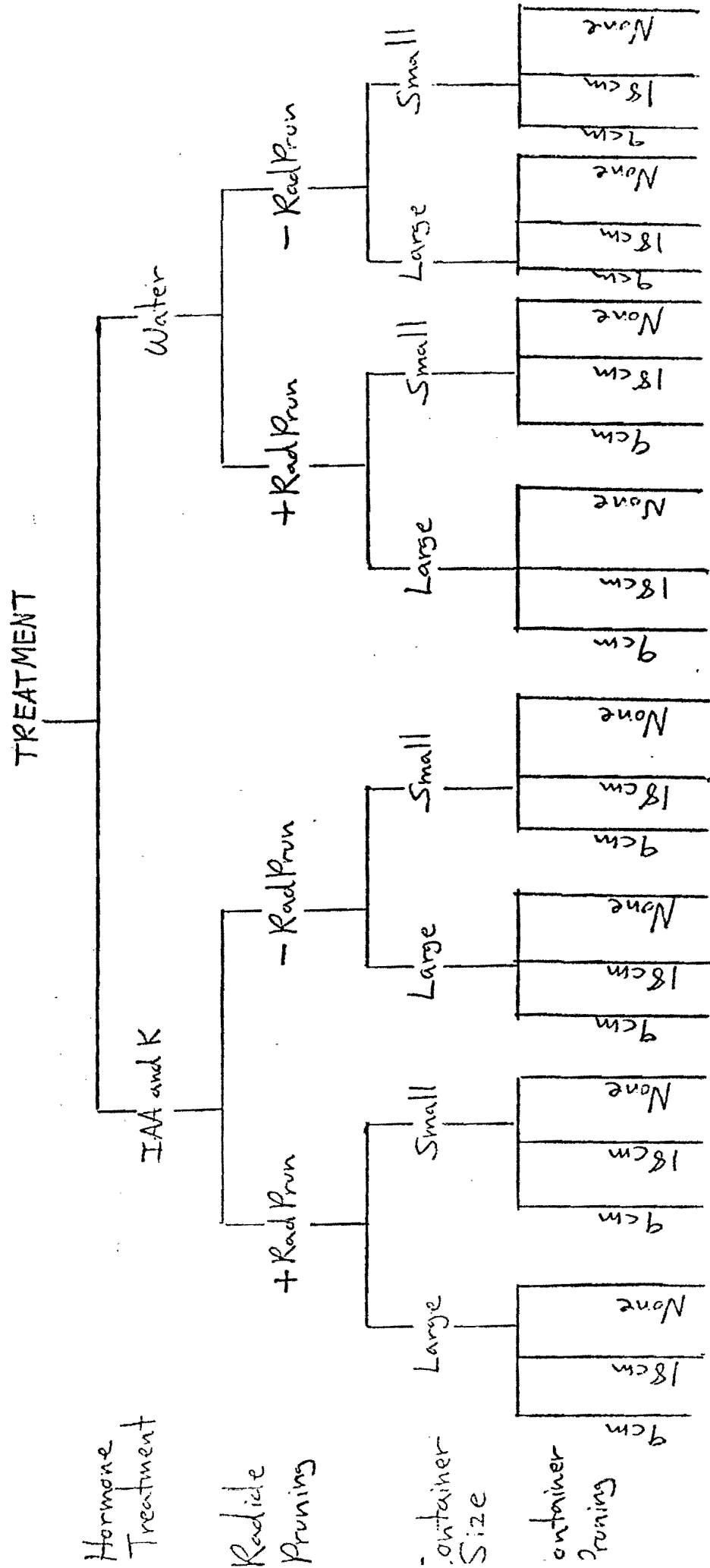
4. Container prunings

One-third of the planted seedlings were root-tip pruned when their root tips beyond 9cm. from the container tops, one-third of the seedlings were pruned when their root tips were beyond 18cm. from the container tops, and the remaining third were not container pruned.

C. Integration of Treatments

From the above design of dichotomous hormone, radicle pruning, and container size treatments, and trichotomous container pruning treatments, an integrated schematic of all treatments was devised and assigned to the seedlings. Each seedling received one of two hormone treatments, one of two radicle pruning treatments, was grown in one of two container sizes, and was given one of three root pruning treatments, resulting in a treatment matrix $2 \times 2 \times 2 \times 3$, or 24 possible treatment combinations (refer to page 7).

INTEGRATION OF TREATMENTS



Section II: Experimental Procedure

A. Seed preparation and hormone treatment (Sept.22 - Dec.11)

White oak seed was collected on September 22 at the Mohican Hills Golf Course near Wooster, Ohio, and was stored in plastic bags at 3°C. until December 10. On December 10, 500 seeds were put in a vacuum chamber with a solution of 100ppm. IAA and 0.1ppm. kinetin, and 500 seeds were put in a vacuum chamber with water as a control soak. Both treatment soaks lasted for 16 hours. On December 11, the soaked seeds were planted in flats of moistened vermiculite in a greenhouse with natural light. The seeds were grouped in separate flats according to the hormone treatment they received.

B. Radicle pruning and seed planting (Dec.16)

A large majority of the seeds were germinated, with radicles averaging about 2.5cm. \pm 1.0cm. Radicles of hormone treated seeds appeared to be slightly poorer in quality. A minority of the seeds had double embryos and were not used, and a small number of seeds were infected with a white fungus.

Half of the hormone treated seeds planted and half of the water treated seeds planted were radicle pruned, with about 5mm. of the radicle tip removed.

A total of 672 seeds were planted. 60 of these were planted in clear plastic tubes to serve as indicators of root length position for timing the container prunings. The remaining seeds were planted in the two sizes of containers. All containers and tubes were labeled as to which type of hormone treated and radicle pruned treated seed they contained.

C. Container prunings (Dec.26 - Jan.12)

The timing of the container prunings was determined by the seedlings in the clear plastic tubes, which allowed root lengths to be observed. When ~~a majority~~^{all} of the tube seedlings of a certain hormone and radicle pruned type had root tips extended beyond 9cm., a container pruning was performed on container seedlings of the corresponding hormone and radicle pruned type. Likewise, when ~~a majority~~^{all} of tube seedlings had roots extended beyond 18cm., an 18cm. level pruning was performed on container seedlings.

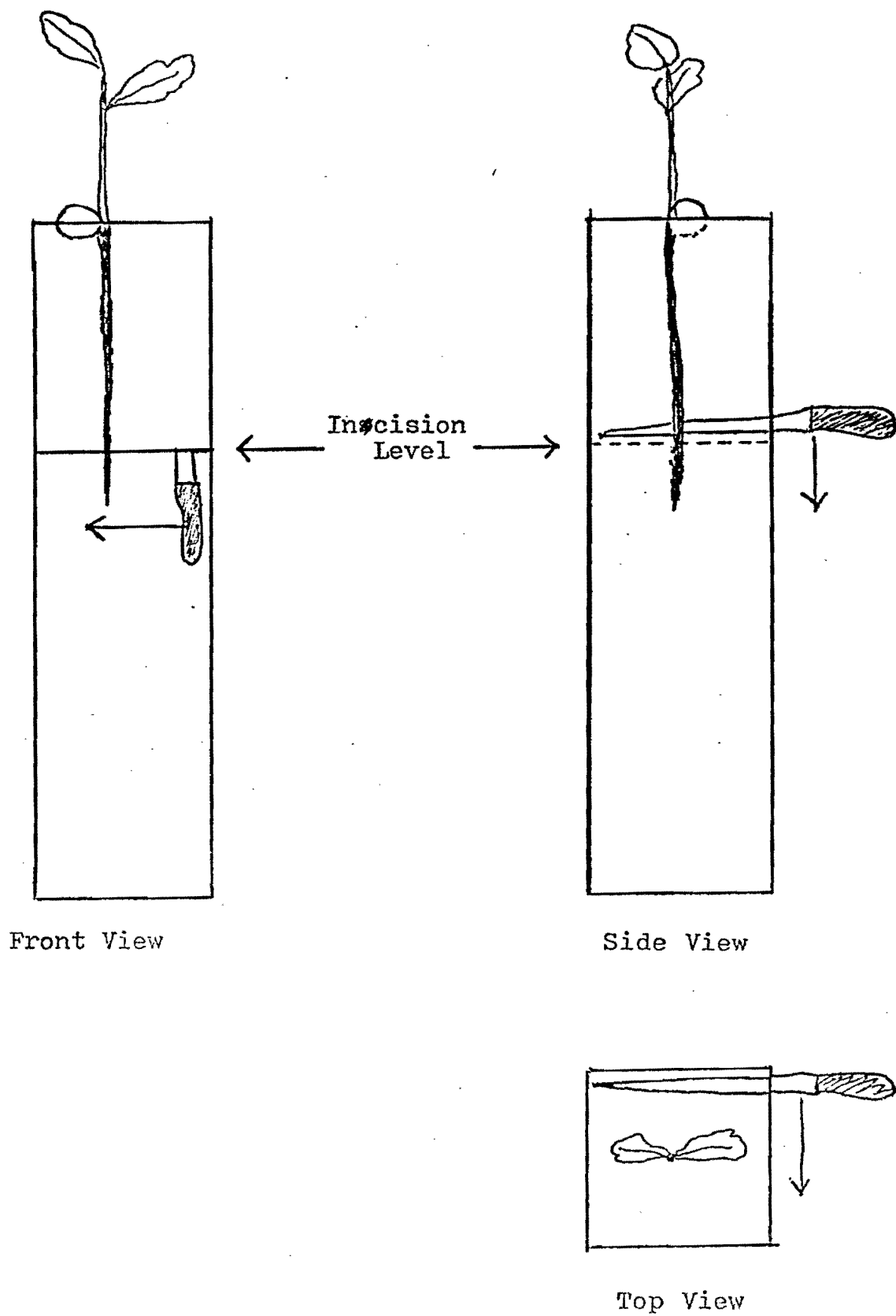
The pruning technique consisted of cutting through one side of the container walls of the selected seedlings with a surgical knife, and passing the knife blade across the horizontal plane of incision. (refer to page 10).

1. Container pruning 9cm. level (Dec.26 and Jan.2)

- a) Container and tube trees which were not radicle pruned were container pruned on Dec. 26.
- b) Container and tube trees which were radicle pruned were container pruned on Jan. 2.

2. Container pruning 18cm. level (Jan.7 and Jan.12)

- a) Container and tube trees not radicle pruned were container pruned on Jan. 2.
- b) Container and tube trees which were radicle pruned were pruned on Jan.12.

Container Pruning Technique

Section III: Treatment Analyses

The effects of the different combinations of hormone treatments, radicle prunings, container sizes, and container prunings on seedling growth were determined by harvesting samples of seedlings of each treatment combination from their containers at three intervals during the growth period in the greenhouse and measuring various root and shoot size parameters at each interval. From these measurements, general growth curves were constructed for the seedlings, and statistical inferences about specific treatments were made.

A. Seedling harvest intervals

Three seedling harvests were made during the growth period after all cultural treatments were applied and had time to effect seedling growth. The first harvest was made January 22, 10 days after the last container pruning. The second harvest was made on February 13 and the third harvest was made on March 23. These harvests represented 37, 59, and 93 days of seedling growth in containers.

B. Sample sizes of harvested seedlings

Each of the 24 treatment combinations were represented by 21 seedlings in containers, so 7 seedlings of each treatment combination were harvested and analyzed at each of the three intervals.

C. Measurements of the harvested seedlings

1) Shoot measurements

- | | |
|---------------------|---------------------------|
| a) Total height | e) Leaf dry weight |
| b) Number of stems | f) Cotyledon dry weight |
| c) Number of leaves | g) Top (shoot) dry weight |
| d) Stem dry weight | |

2) Root measurements

- a) Longest root length
- b) Number of main roots
- c) Number of regenerated roots after pruning
- d) Number of main roots 6.6cm below the root collar
- e) Number of main roots 13.2 cm below the root collar
- f) Number of main roots 19.8 cm below the root collar
- g) Dry weight of root section from 0-6.6cm.
- h) Dry weight of root section from 6.6-13.2cm.
- i) Dry weight of root section from 13.2-19.8cm
- j) Dry weight of root section beyond 19.8cm
- k) Total root dry weight

3) Total tree measurements

- a) Total tree dry weight
- b) Root-shoot dry weight ratio

D. Data

Root and shoot measurement data for each harvest was processed at the Statistics Laboratory, OARDC, and computer printouts were produced which listed each tree seedling's cultural treatment and corresponding root and shoot measurements, presented one-way analysis of variance tables for each growth parameter, and presented Duncan's Multiple Range Test results for those treatments with F probabilities of 5% or less. From this information, the following summaries were made.

E. Data Summaries

1) Growth curves

Growth curves of each root and shoot growth parameter were made, based on the average measurements of all 168 container seedlings harvested at each interval, to illustrate general trends of seedling growth and to serve as standards when comparing growth trends effected by specific treatments.

(Refer to Table 1 and Graphs 1-5 in Appendix)

2) Significant difference tables

Summary tables were made showing the type and number of root and shoot growth parameters each treatment^{variation} significantly effected at the 5% and 10% levels at each seedling harvest.

(Refer to Tables 2-4 in Appendix)

3) Effective treatment tables

Summary tables were made showing how specific treatments effected numerous root and shoot growth parameters consistently within and between harvests at the 5% level of significance.

(Refer to Tables 5-8)

From these data summaries, the following conclusions were made about the tested treatments and overall seedling growth.

Conclusions

The growth curves of root and shoot size parameters, constructed from the three seedling harvests, revealed several trends of seedling growth. Total shoot dry weight, shoot height, and leaf dry weight increased at slower growth rates after the second harvest. Stem dry weights increased at a steady rate during the growth period, however, and the number of stems and leaves remained relatively constant. Cotyledon dry weights steadily decreased during the growth period. Total root dry weight and all of the individual root section dry weights increased at a faster rate after the second harvest. Root section growth rates were about equal after the second harvest, except for the 19.8cm section which had a faster growth rate. The average dry weight of the 0-6.6cm root section was highest during the growth period and that of the 6.6cm-13.2cm section was second highest. The dry weights of the 13.2-19.8cm and 19.8cm root sections were about equal until the second harvest, when the 19.8cm section became third highest in dry weight. The total number of main roots decreased during the growth period and so did those at the 6.6cm level. The number of main roots at the 19.8cm level increased and the number of roots at the 13.2cm level remained fairly constant. Total seedling dry weight increased at a faster rate after the second harvest due to faster root growth, and the root-shoot ratios increased throughout the growth period.

The significant difference tables for the three harvests showed that almost every source of treatment variation effected some growth parameter at one time or another. Some sources of variation effected several growth parameters at the 5% level and were studied closer to determine what their effects of growth were. Some of these treatment variations were found to produce inconsistent effects on related growth parameters at the same harvest interval while other variations produced inconsistent effects on the same growth parameter at different harvest intervals. Therefore, only those treatment variations which effected several related growth parameters with consistency at the 5% level of significance were analyzed further. These sources of variation were radicle pruning, container size, and the IAA and K hormone treatments.

The treatment variation of radicle pruning effected several root and shoot parameters at all three harvest intervals. The effect of radicle pruning on root development was an increased number of total main roots, number of roots at all levels, and number of regenerated roots throughout the growth period. Pruning reduced root dry weight at all levels, however, for the first two harvests. Root weights between pruning treatments at the third harvest were about equal. The effect of radicle pruning on shoot development was an increase in shoot height, stem dry weight, and leaf dry weight throughout the growth period. Total tree dry weight was not effected, but pruning resulted in lower root-shoot ratios, because of increased shoot growth.

The treatment variation of container size did not produce effects until the third harvest, but this harvest showed that the larger containers increased total root dry weights and dry weights of all root sections except the 13.2-19.8cm section. The effect of the large containers on shoot development was an increased dry weight of the total shoots, stems, and leaves. Overall, the larger containers did produce larger seedlings, but root-shoot ratios were lower.

The effects of IAA and K were evident only at the third harvest and this treatment effected shoot growth only. Total shoot weight and height were increased, along with stem and leaf dry weights. Thus, the root-shoot size ratios were decreased.

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In summary, the use of radicle pruning and large containers for more extensive root development in White oak seedlings looks promising, but no significant interactions between the two were found, and ^{the} goal of increasing root-shoot ratios with these methods was not reached.

IAA and K treatments and container pruning methods showed no advantages for root development, and none of the higher level interactions between treatments produced consistent significant growth responses.

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APPENDIX

[illegible]

Average Stem Length (Total Height) of All Seedlings as a Function of Growth Period in Containers

Stem Length in Millimeters

110
109
108
107
106
105
104
103
102
101
100
(mm)

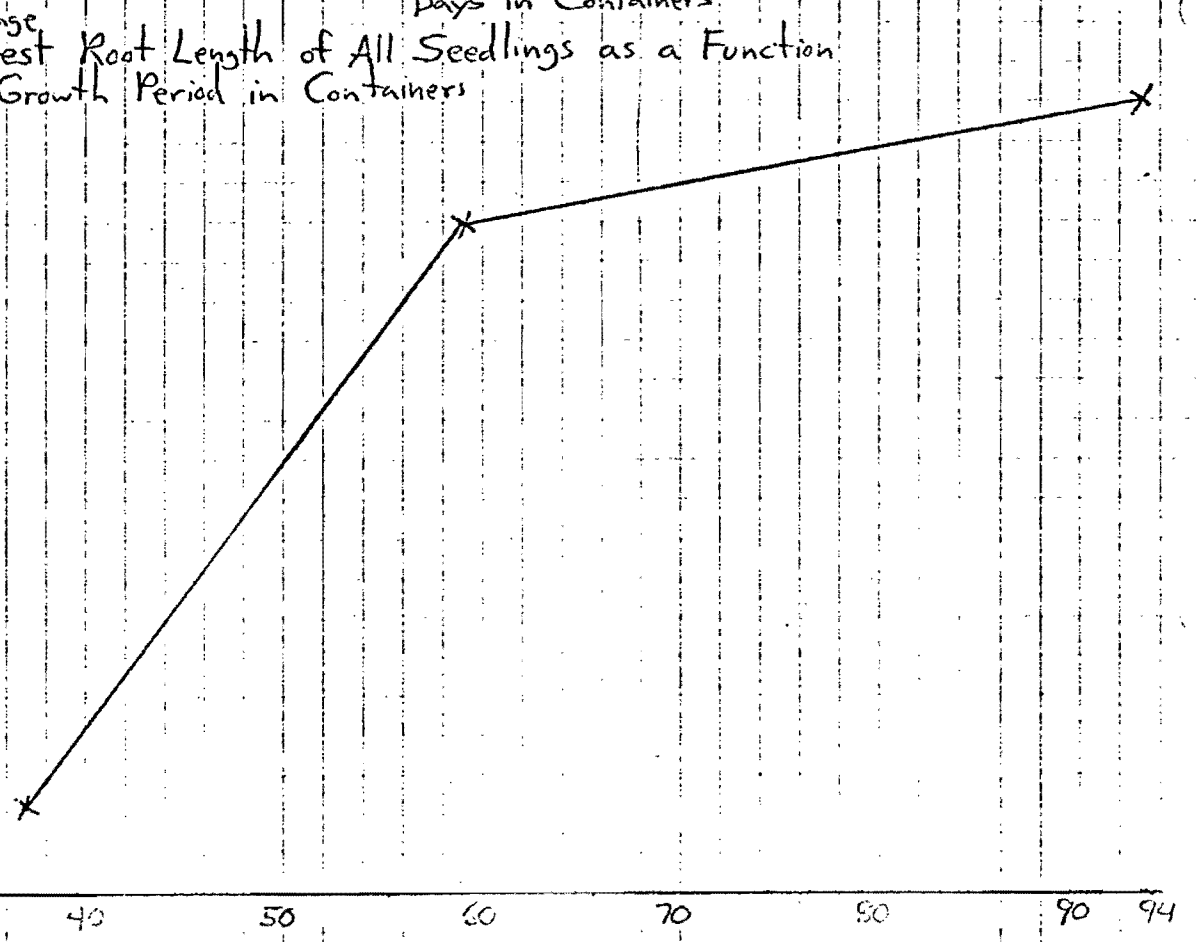
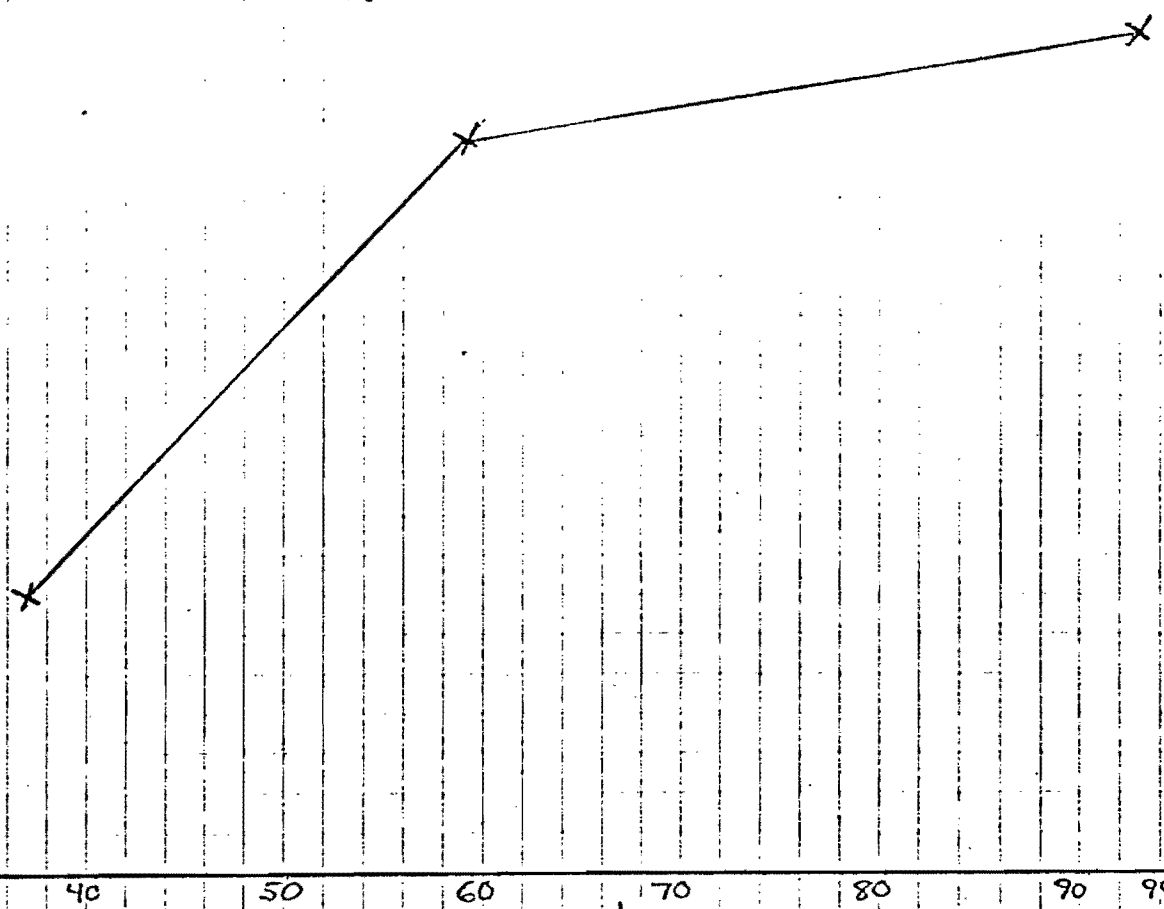
Days in Containers

Average Longest Root Length of All Seedlings as a Function of Growth Period in Containers

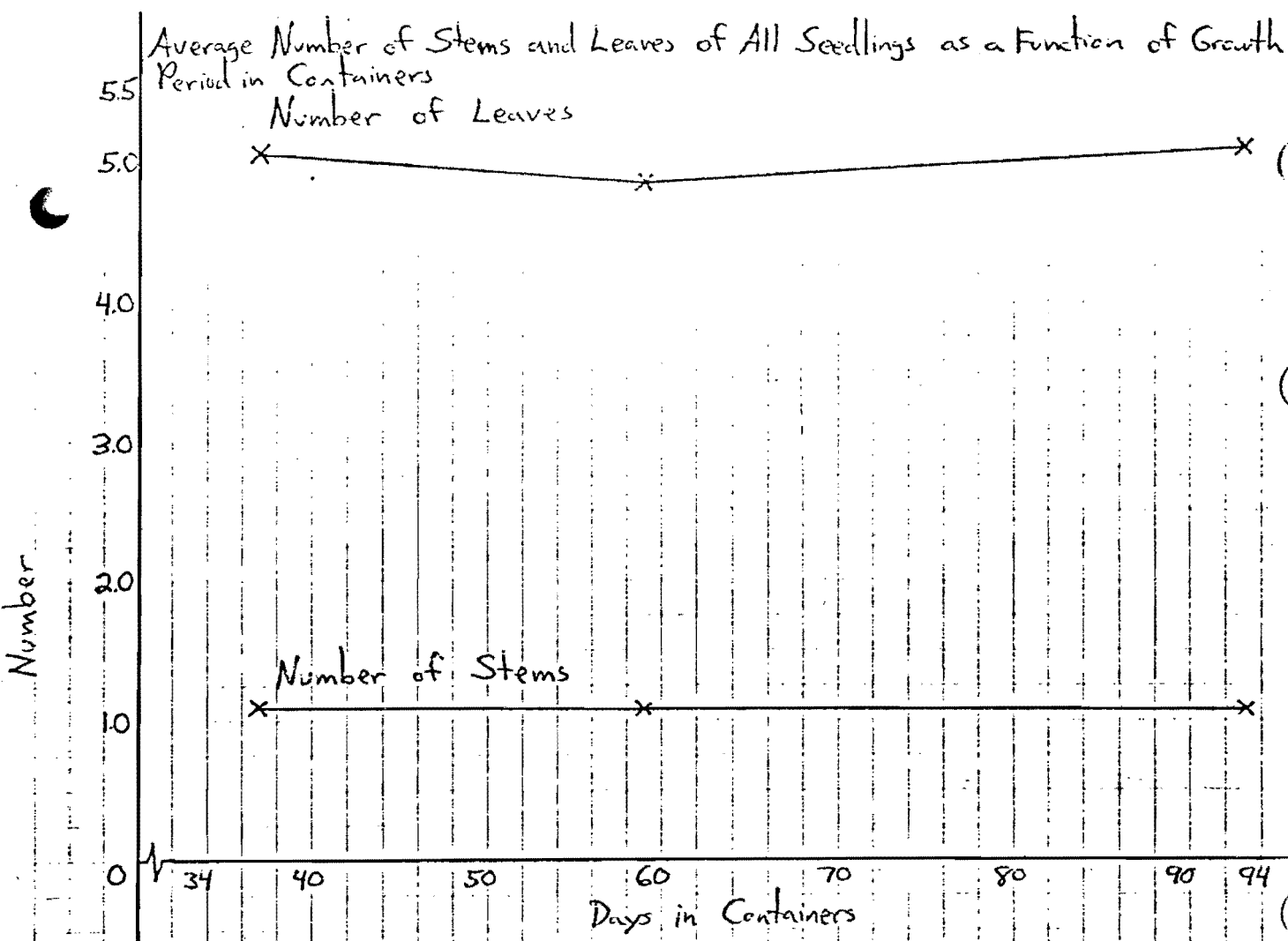
Root Length in Millimeters

400
390
380
370
360
350
340
330
320
310
300
(mm)

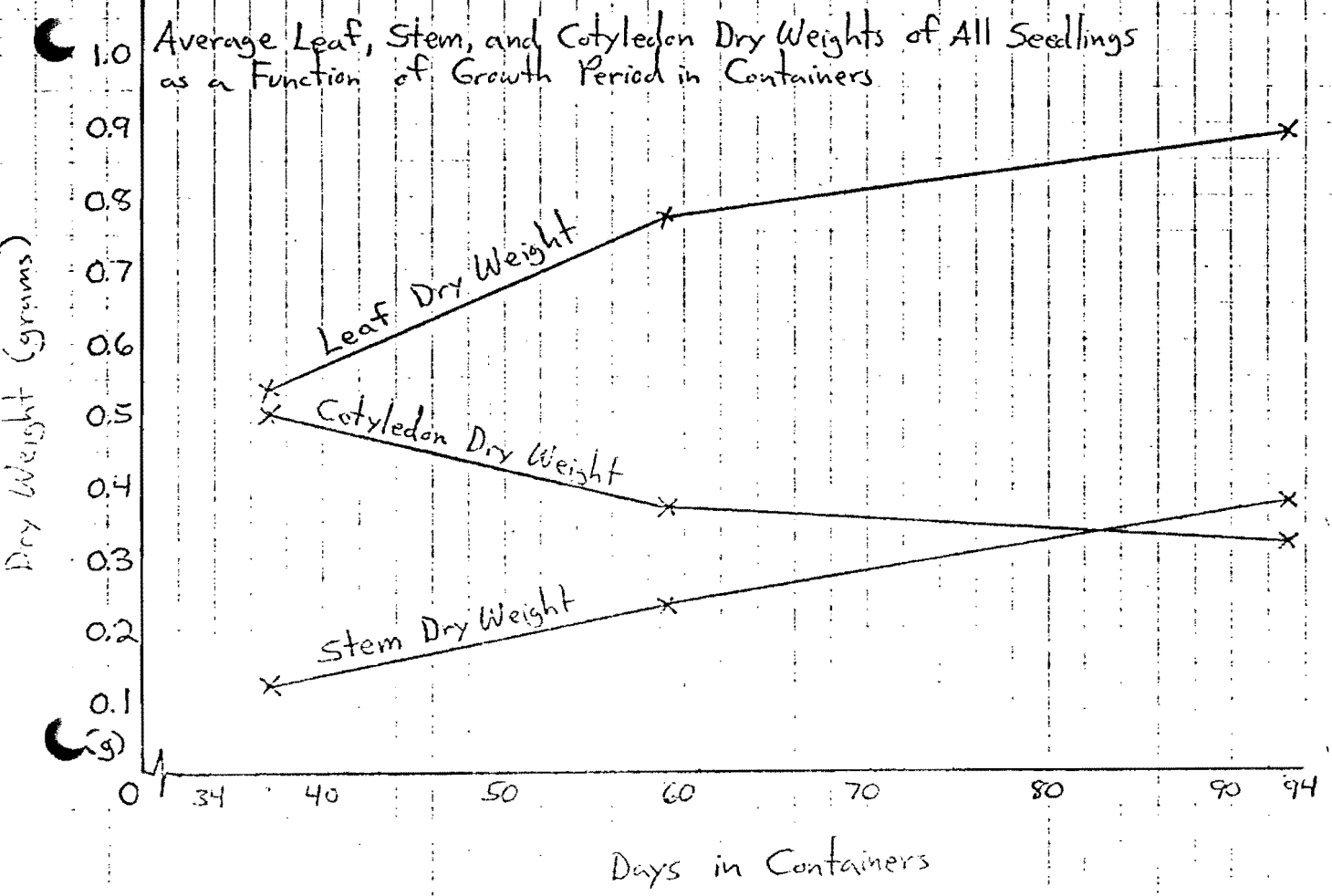
Days in Containers



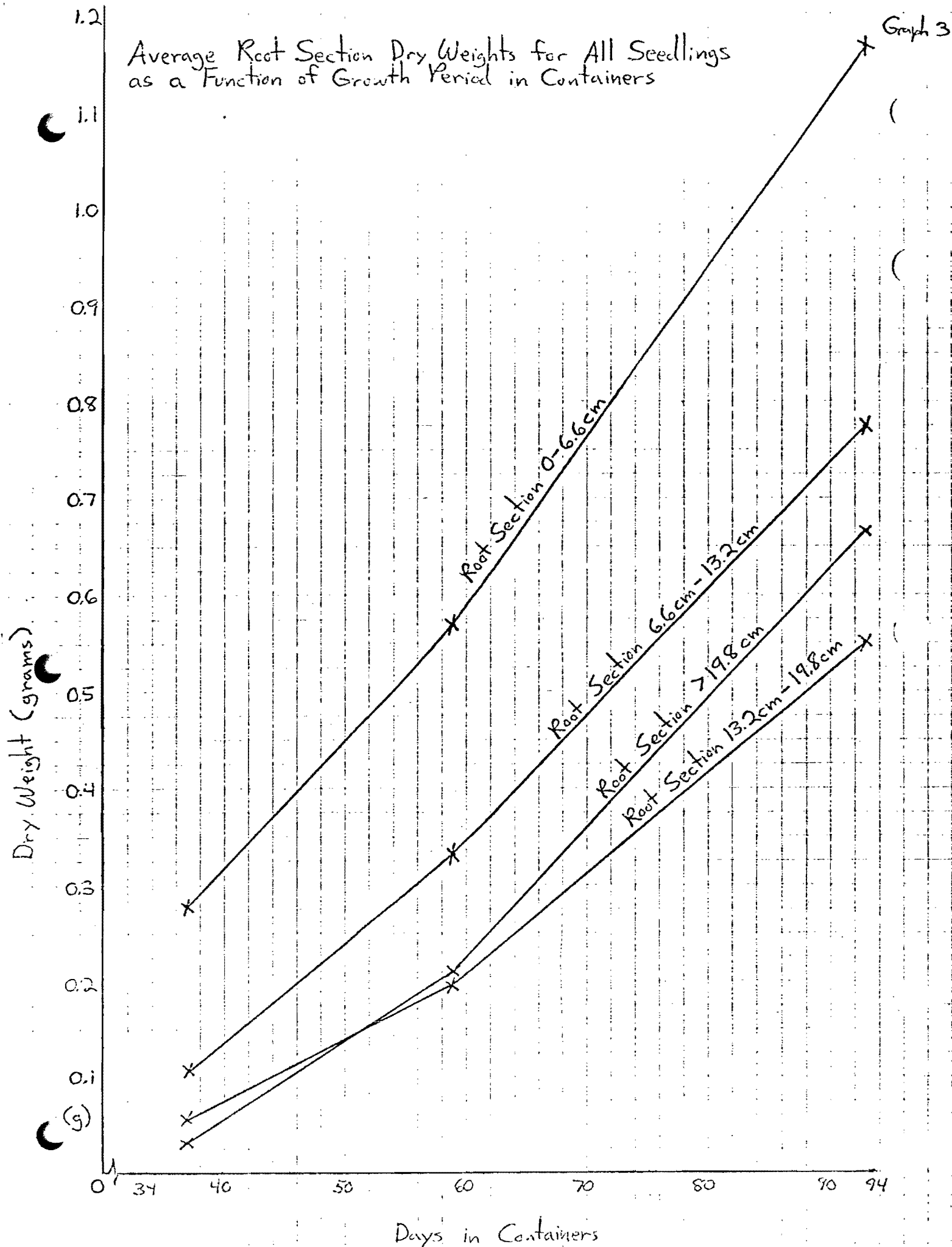
Average Number of Stems and Leaves of All Seedlings as a Function of Growth Period in Containers
Number of Leaves



Average Leaf, Stem, and Cotyledon Dry Weights of All Seedlings as a Function of Growth Period in Containers

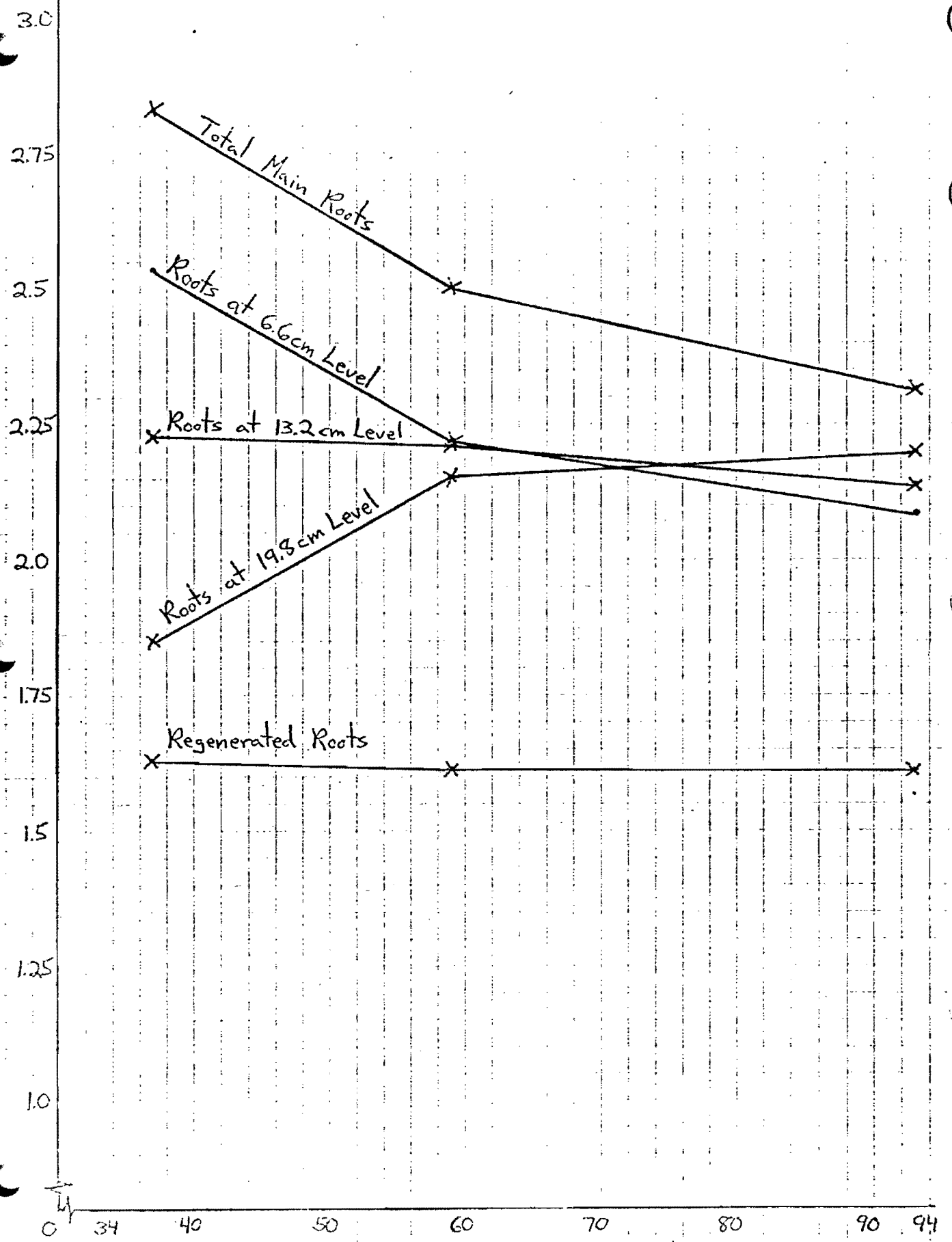


Average Root Section Dry Weights for All Seedlings
as a Function of Growth Period in Containers

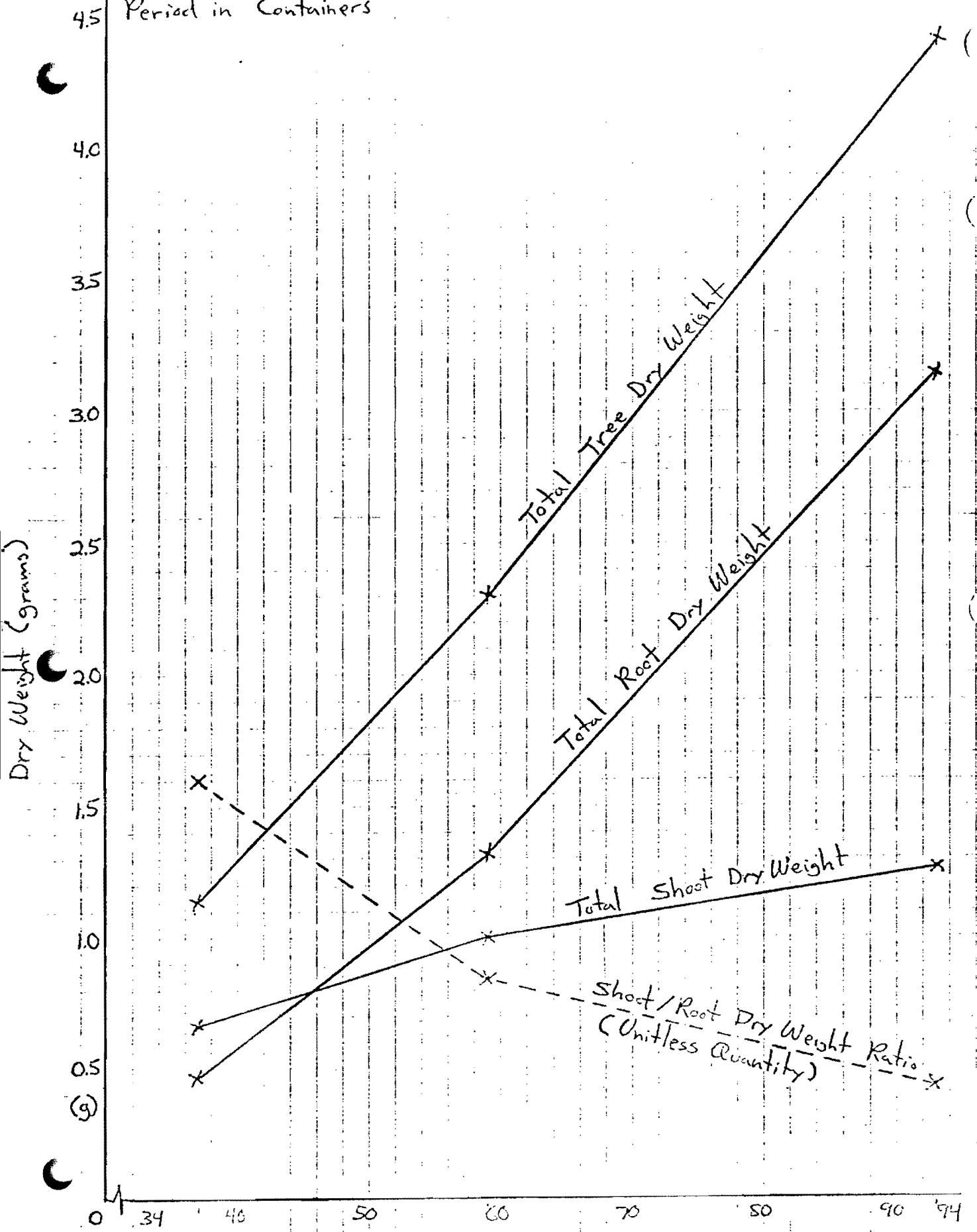


Average Number of Main Roots of All Seedlings as a Function of Growth Period in Containers

Graph 4



Average Total Root, Shoot, and Tree Dry Weights, and Shoot/Root Dry Weight Ratios of All Seedlings as a Function of Growth Period in Containers Graph 5



SIGNIFICANT DIFFERENCES

Table 2

HARVEST 1

ROOT AND SHOOT PARAMETERS

Source of Variation	TOT - HT	N - STEMS	N - LEAVES	LONG-ROOT	N-MA-ROOTS	N-REG-ROOTS	R-6.6	R-13.2	R-19.8	STEM-DWT	LEAVES-DWT	COT-DWT	TOP-DWT	R6.6-DWT	R13.2-DWT	R19.8-DWT	R>19.8-DWT	TROOT-DWT	TREE-DWT	R/S RATIO
PRUN (RAD)	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
HORM																				
HORMxPRUN				*							*		*						*	
C-PRUN	X		X	X	X	X		X		X	X		X		X		X			X
C-PRUN • PRUN	*				X	X	X	*		*				*						
C-PRUN • HORM														X	X			*		
C-PRUN • HORM • PRUN	X				*			*	*					*	X					X
C-SIZE				X																
C-SIZE • PRUN																				
C-SIZE • HORM	*		*																	
C-SIZE • HORM • PRUN									*						X		*			
C-SIZE • C-PRUN										*	X		X						X	
C-SIZE • C-PRUN • PRUN						X				*	X		X						*	
C-SIZE • C-PRUN • HORM																				
C-SIZE • C-PRUN • HORM • PRUN																				
REPLICATIONS	*			*													X			

HARVEST 2

ROOT AND SHOOT PARAMETERS

Source of Variation	TOT - HT	N - STEMS	N-LEAVES	LONG-ROOT	N-MA-ROOTS	N-REG-ROOTS	R-6.6	R-13.2	R-19.8	STEM-DWT	LEAVES-DWT	COT-DWT	TOP-DWT	R6.6-DWT	R13.2-DWT	R19.8-DWT	R>19.8-DWT	TROOT-DWT	TREE-DWT	R/S RATIO
PRUN (RAD)	X		X		X	X	X	X	X	X	X	X	X	X	X	X		X		X
HORM			X		*		*													
HORMxPRUN					X	X		X	*											
C-PRUN						X	X	X							*		X			X
C-PRUN•PRUN						X									X	X				X
C-PRUN•HORM											X		X	X	X	X		X	X	
C-PRUN•HORM•PRUN																				
C-SIZE				X				*	*	X	X	*	X							X
C-SIZE•PRUN																				
C-SIZE•HORM																*	*			
C-SIZE•HORM•PRUN							*	X	X	*	X		X	*				X	X	
C-SIZE•C-PRUN			X			X		X	X			X								
C-SIZE•C-PRUN•PRUN				X										*	X		X	X		
C-SIZE•C-PRUN•HORM				*	X		*	X	*							X				
C-SIZE•C-PRUN•HORM•PRUN					X		X	*	X											
REPLICATIONS								X			X		X							

HARVEST #3

ROOT AND SHOOT PARAMETERS

[illegible]

EFFECTS OF RADICLE PRUNING ON GROWTH PARAMETERS

Growth Parameters		Radicle pruning 1=no, 2=yes	HARVEST 1 (37days)	HARVEST 2 (59days)	Harvest 3 (93days)			
<u>ROOT</u> Long-root (mm)	1.		314.1	371.9	387.7	NUMBERS IN BOXES ARE SIG. DIFF. AT 5% LEVEL		
	2		288.2	377.4	392.1			
N-Ma-roots	1		1.57	1.39	1.49			
	2		4.08	3.61	3.15			
N-Reg roots	1		1.10	1.06	1.17			
	2		2.15	2.17	2.05			
R6.6	1		1.23	1.10	1.19			
	2		3.86	3.35	2.96			
R13.2	1		1.42	1.35	1.39			
	2		3.04	3.08	2.89			
R19.8	1		1.45	1.43	1.54			
	2		2.24	2.87	2.87			
R6.6 dwt (g)	1		0.33	0.61	1.16			
	2		0.22	0.54	1.18			
R13.2 dwt (g)	1		0.13	0.37	0.82			
	2		0.08	0.29	0.68			
R19.8 dwt (g)	1		0.07	0.21	0.54			
	2		0.04	0.18	0.56			
R19.8 dwt (g)	1		0.04	0.20	0.64			
	2		0.02	0.21	0.69			
Root dwt (g)	1		0.57	1.39	3.17			
	2		0.36	1.23	3.11			

EFFECTS OF RADICLE PRUNING ON GROWTH PARAMETERS (contd.)

Pruning treat. 1= no. 2= yes	Radicle pruning 1=no, 2=yes	HARVEST 1 (37 days)	HARVEST 2 (59 days)	HARVEST 3 (93 days)				
Growth Parameters								
<u>SHOOT</u>								
Tot-Ht (mm)	1	98.6	102.2	101.4	NUMBERS IN BOXES ARE SIG...DIFF. AT 5% LEVEL			
	2	105.4	113.3	116.6				
Stem dwt (g)	1	0.11	0.21	0.33				
	2	0.13	0.25	0.42				
Leaves dwt (g)	1	0.50	0.70	0.80				
	2	0.57	0.84	0.97				
N-Leaves	1	4.74	4.67	4.75				
	2	5.31	5.06	5.37				
Cot dwt (g)	1	0.44	0.32	0.31				
	2	0.57	0.41	0.33				
Top dwt (g)	1	0.62	0.91	1.12				
	2	0.70	1.10	1.39				
<u>TOTAL TREE</u>								
Tree dwt (g)	1	1.19	2.30	4.32				
	2	1.06	2.31	4.50				
R/S ratio	1	0.89	1.44	2.68				
	2	0.48	1.04	2.14				

EFFECTS OF CONTAINER SIZE ON GROWTH PARAMETERS

Growth Parameters		Container size 1=small 2=large	HARVEST 1 (37days)	HARVEST 2 (59days)	HARVEST #3 (93days)		
<u>ROOT</u> Long-root (mm)	1		292.2	361.0	364.9	NUMBERS IN BOXES ARE SIG. DIF. AT 5% LEVEL	
	2		310.1	388.2	414.8		
R6.6 dwt (g)	1		0.29	0.57	1.12		
	2		0.27	0.58	1.22		
R19.8 dwt (g)	1		0.05	0.19	0.51		
	2		0.06	0.20	0.59		
R>19.8 dwt (g)	1		0.03	0.20	0.60		
	2		0.03	0.22	0.74		
Troot dwt (g)	1		0.48	1.31	2.96		
	2		0.46	1.31	3.31		
<u>SHOOT</u> Tot-ht (mm)	1		99.8	105.5	103.5		
	2		104.2	110.0	114.5		
Stem dwt (g)	1		0.12	0.22	0.34		
	2		0.12	0.24	0.40		
Top dwt (g)	1		0.65	0.94	1.13		
	2		0.67	1.06	1.39		
Leaves dwt (g)	1		0.53	0.73	0.79		
	2		0.55	0.81	0.98		
<u>TOT. TREE</u> Tree dwt (g)	1		1.12	2.24	4.11		
	2		1.13	2.37	4.70		
R/S ratio	1	0.65	1.54	1.30	0.77	2.50	0.40
	2	0.61	1.55	1.14	0.88	2.27	0.44

Growth Parameters

NUMBERS IN
BOXES ARE
SIG. DIG.
AT 5% LEVEL